LIVER INFLAMMATION EXACERBATES INTRASPINAL TISSUE LOSS AFTER SPINAL CORD INJURY

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Background

Traumatic spinal cord injury (SCI) is a devastating condition that significantly reduces a patient's sensory and motor abilities. SCI can cause multiple organ system dysfunction that contributes to chronic health impairments. Prior work from our lab showed that immune system cells called Kupffer Cells (KCs), which are resident macrophages of the liver, initiate a pro-inflammatory response that persists long after SCI. Additionally, some reports suggest that liver inflammation may exacerbate lesion pathology in the spinal cord after SCI, though this effect has not been directly tested. Therefore we employed a bile duct ligation (BDL) surgical model to induce liver inflammation prior to a thoracic spinal cord contusion to test our hypothesis that inducing liver inflammation before SCI will increase spinal inflammation, lesion pathology, and functional deficits.

Methods

Adult Sprague-Dawley rats received a total BDL and were sacrificed at 1 and 5 days. Fresh liver samples were used to examine pro-inflammatory gene expression. Other rats were given a T8 spinal cord contusion five days after BDL and were sacrificed 28 days post BDL. Control animals received sham BDL surgery followed by T8 contusion. Liver Kupffer cells (KCs) and spinal cord macrophages were examined with OX42 immunohistochemistry. Fresh liver samples were again used to analyze chronic pro-inflammatory gene expression. Lipid accumulation and morphological changes within the liver were examined with Oil Red O and Hematoxylin and Eosin (H&E) staining, respectively. White matter and axonal damage within the spinal cord lesion were visualized with Eriochrome Cyanine (EC) and neurofilament (NF) labeling, respectively. Hindlimb motor function was determined using the Basso, Beattie, and Bresnahan (BBB) locomotor scale.

Funding

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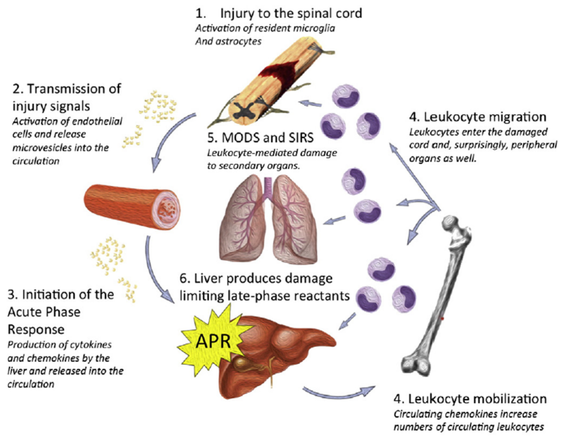
The innate immune system response following SCI



Anthony & Couch (2014)

BDL increases acute pro-inflammatory gene expression in the liver

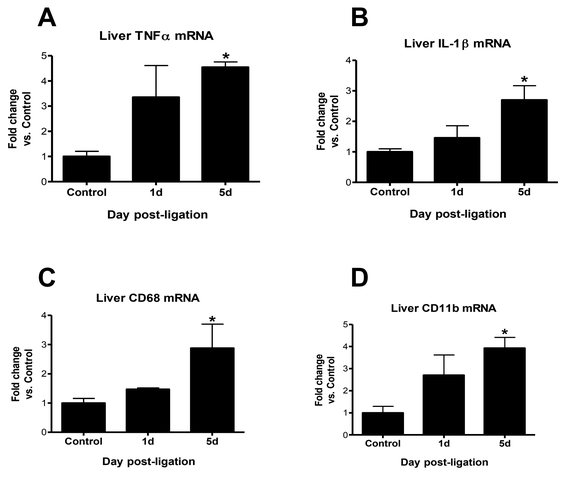


Figure 1: RT-PCR of liver tissue samples at 1 and 5 days post-BDL for **(A)** TNFα, **(B)** IL-1β, **(C)** CD68 and **(D)** CD11b. Expression of all pro-inflammatory genes examined were significantly increased by 5 dpi. *p < 0.05 vs Control.

Liver fat deposition and Kupffer Cell activation are altered after BDL and SCI

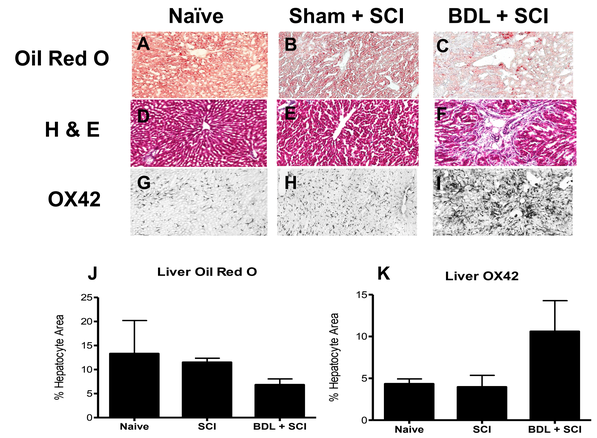


Figure 2: Trends of decreased Oil Red O+ staining showing fat deposition **(A-C, J)**, H & E for morphological changes **(D-F)** and increased OX42+ Kupffer Cell activation **(G-I, K)** 28 days post BDL and 23 days post SCI.

Chronic upregulation of pro-inflammatory gene expression in the liver following BDL and SCI

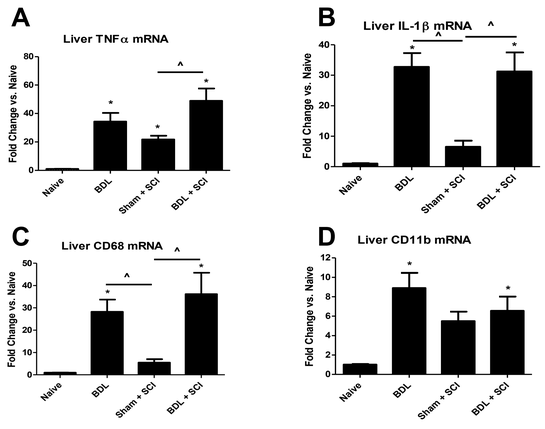


Figure 3: RT-PCR of liver tissue samples at 23 days post-SCI and 28 days post-BDL for **(A)** TNFα, **(B)** IL-1β, **(C)** CD68 and **(D)** CD11b. BDL and BDL + SCI induced significant chronic increases in all pro-inflammatory genes in the liver compared to naïve animals. *p < 0.05 vs. Naive., ^p < 0.05.

Spinal contusion preceded by hepatic inflammation leads to increased tissue damage

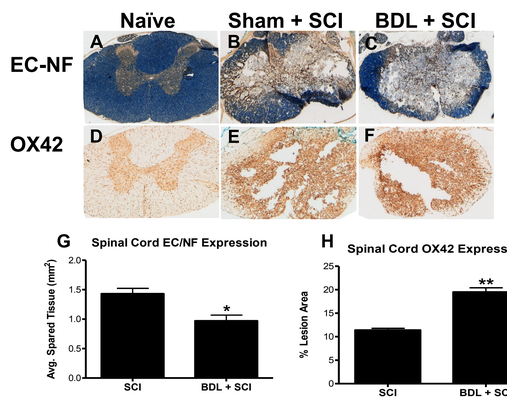


Figure 4: (A-C, G) Myelin-axon (EC/NF) staining revealed increased lesion pathology and tissue loss in the spinal cord 28 days post-BDL and 23 days post-SCI. **(D-F, H)** Similarly, **OX42** revealed significantly increased macrophage and microglia activation. *p<0.05 vs. SCI., **p<0.01 vs. SCI.

Hepatic inflammation does not affect locomotor recovery

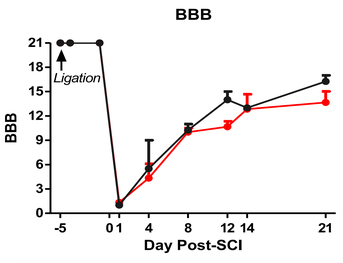


Figure 5: Trend of decreased hindlimb locomotor scores for the BDL + SCI group compared to the SCI group.

Conclusions

Pathological effects of SCI are exacerbated when preceded by induced liver inflammation.

Significant findings include:

- Inflammation-induced molecular, cellular and morphological changes in the liver
- Potential changes in SCI lesion size and increased spared tissue